Design criteria for developing low-resource magnetic bead assays using surface tension valves

Nicholas M. Adams,[1,2] Amy E. Creecy,[1] Catherine E. Majors,[1,a)] Bathsheba A. Wariso,[1,b)] Philip A. Short,[1] David W. Wright,[2] and Frederick R. Haselton[1,c)]
[1]*Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee 37235, USA*
[2]*Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235, USA*

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Many assays for biological sample processing and diagnostics are not suitable for use in settings that lack laboratory resources. We have recently described a simple, self-contained format based on magnetic beads for extracting infectious disease biomarkers from complex biological samples, which significantly reduces the time, expertise, and infrastructure required. This self-contained format has the potential to facilitate the application of other laboratory-based sample processing assays in low-resource settings. The technology is enabled by immiscible fluid barriers, or surface tension valves, which stably separate adjacent processing solutions within millimeter-diameter tubing and simultaneously permit the transit of magnetic beads across the interfaces. In this report, we identify the physical parameters of the materials that maximize fluid stability and bead transport and minimize solution carryover. We found that fluid stability is maximized with $\leq$0.8 mm i.d. tubing, valve fluids of similar density to the adjacent solutions, and tubing with $\leq$20 dyn/cm surface energy. Maximizing bead transport was achieved using $\geq$2.4 mm i.d. tubing, mineral oil valve fluid, and a mass of 1-3 mg beads. The amount of solution carryover across a surface tension valve was minimized using $\leq$0.2 mg of beads, tubing with $\leq$20 dyn/cm surface energy, and air separators. The most favorable parameter space for valve stability and bead transport was identified by combining our experimental results into a single plot using two dimensionless numbers. A strategy is presented for developing additional self-contained assays based on magnetic beads and surface tension valves for low-resource diagnostic applications. [http://dx.doi.org/10.1063/1.4788922]

I. INTRODUCTION

Magnetic bead-based methods have been developed for a variety of biological and biochemical applications such as biomolecule extraction, amplification, and detection, because, in part, they enhance the flexibility and simplicity of the solid phase assay format.[1–6] Perhaps one of the clearest examples of the utility of magnetic beads is the development of automated, high-throughput, parallel extraction of nucleic acids from biological samples in a 96-well plate format,[7] a process that would be too cumbersome or inefficient using a traditional column-based solid phase format. Despite their success, magnetic bead-based assays still generally require relatively complex procedures that involve dispensing multiple solutions or transferring the beads between solutions. For settings where trained personnel are not available or specialized laboratory equipment is too cumbersome for the application, such as in point-of-care

a)Current address: Department of Bioengineering, Rice University, Houston, Texas 77005, USA.
b)Current address: National Institutes of Health, Bethesda, Maryland 20892, USA.
c)Author to whom correspondence should be addressed. Electronic mail: rick.haselton@vanderbilt.edu.

diagnostics, extensive solution handling can reduce assay efficacy and, in many cases, is not feasible. Because these obstacles are faced in low-resource settings, simple, self-contained formats for magnetic bead-based applications are highly desirable.

The development of multiphase fluidic systems for fluid separation and manipulation has also been used for simplifying and automating chemical and biological assays. In microfluidic systems, controlled fluid-fluid interfaces and microencapsulation of assay components and reagents have been applied to a variety of methods, including large-scale parallelization of chemical screening and high-throughput nucleic acid sequencing.[8] Multiphase microfluidic systems feature robust fluid separation and permit controlled manipulation of assay reagents. These characteristics make multiphase microfluidics a desirable platform for developing technologies to be used in low-resource settings.

We have recently exploited the simplicity of magnetic bead-based assays and the robustness of multiphase fluidics to develop methods for the extraction of RNA, DNA, or protein biomarkers in a simple, self-contained format suitable for use in a low-resource environment.[9,10] The analogous commercial kits for nucleic acid or protein extraction require centrifugation and extensive solution handling or pipetting, whereas the self-contained assays we have developed have a much simpler user interface. The assay is carried out within a single length of 1.6 mm inner diameter (i.d.) tubing containing pre-arrayed processing solutions and magnetic beads (Figure 1). The biological sample is added to the first processing solution through the end of the tube using a transfer bulb or by syringe injection though the tubing wall, and then functionalized beads are mixed with the sample using an externally applied magnetic field to selectively capture the biomarker of interest. As the beads are moved from one solution to the next through surface tension valves, the valves maintain their integrity and no solution intermixing occurs. To ensure that the magnetic beads mix properly, the magnetic field is moved back and forth along the length of the tube to disperse the beads throughout the solution.

Surface tension valves are fluids that are immiscible with processing solutions and prevent adjacent solutions from intermixing when arrayed within millimeter-diameter tubing. The valve mechanism is established by selective passage of magnetic beads at the surface tension valve interface. Magnetic beads under the influence of a sufficiently strong magnetic field gradient traverse the immiscible phase when a sufficient mass of beads is gathered at the interface (Figure 2). The surface tension valve format inverts the classical solid phase assay format by immobilizing the assay solutions and making the solid phase the movable entity. The advantages of this format are in its simplicity; using preloaded assay solutions separated by immiscible surface tension valves, cumbersome liquid handling and dispensing steps are eliminated and the assay is carried out by simply manipulating the transit of magnetic particles through the various processing solutions using a magnetic field. This inverted solid phase format based on magnetic beads and stationary fluids separated by surface tension forces has been developed for a number of applications.[11–15] Other laboratory-based magnetic bead assays, such as enzyme-linked immunosorbent assays (ELISAs) or onbead isothermal polymerase chain reaction (PCR), could potentially benefit from the simplicity and flexibility of this format for applications in low-

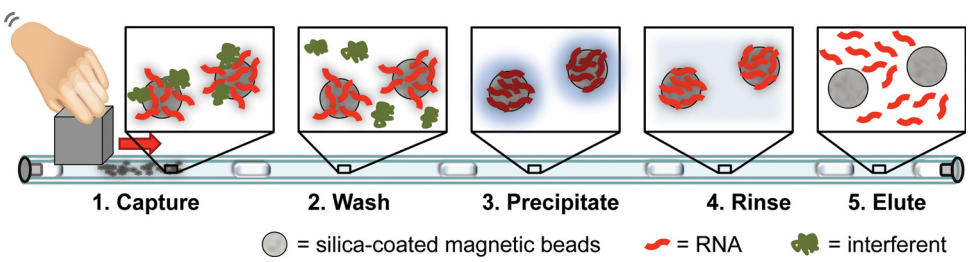

FIG. 1. Illustration of the self-contained format for extraction of RNA biomarkers. Surface tension valves separate unique processing solutions arrayed within a single length of 1.6 mm i.d. tubing. Functionalized magnetic beads used to capture the biomarker of interest are drawn through the surface tension valves into each processing solution using an externally applied magnetic field (i.e., a permanent cube magnet).[8]

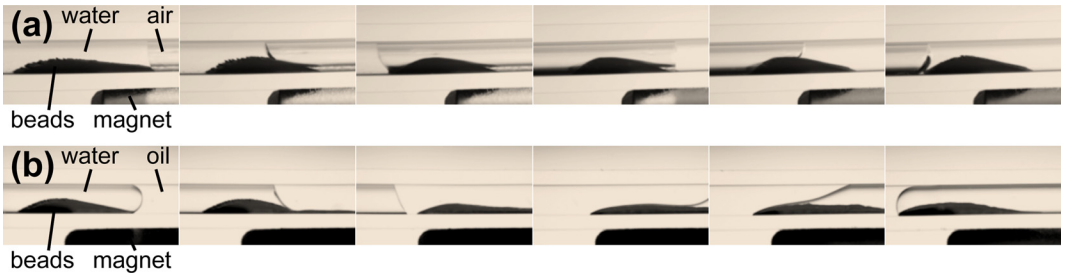

FIG. 2. Selected video images of magnetic beads under the influence of the magnetic field of a permanent magnet moving from one solution to the next through an air surface tension valve (a) or a mineral oil surface tension valve (b) (enhanced online). [URL: http://dx.doi.org/10.1063/1.4788922.1] [URL: http://dx.doi.org/10.1063/1.4788922.2]

resource settings. In this paper, we identify and characterize the key physical design constraints for developing a self-contained format suitable for magnetic bead-based assays.

II. MATERIALS AND METHODS

A. Materials

Tygon R-3603 tubing (0.8, 1.6, 2.4, 3.2, and 4.8 mm i.d.) and Chemfluor fluorinated ethylene propylene (FEP) tubing (1.6 mm i.d.) were purchased from Fisher Scientific. Glass tubing (1.6 mm i.d.) was purchased from the Vanderbilt Glass Shop. Siliconized glass was produced using Sigmacote SL-2 (Sigma-Aldrich) following the manufacturer's protocol. Briefly, the glass was cleaned using a piranha solution (3 H_2SO_4:1 H_2O_2). The glass was then submerged in Sigmacote solution for approximately 1 minute then allowed to dry. The coated glass was then rinsed with water and baked at 100 °C for 1 hour. The silicon coating was validated by a characteristic ∼100° contact angle with water. Dynabeads MyOne Silane beads were purchased from Life Technologies (cat. # 370-02D). MagAttract Suspension E beads were purchased as part of the MagAttract RNA Tissue Mini M48 Kit from Qiagen (cat. # 959236), and AccuBead beads were purchased from Bioneer Corporation (cat. # TS-1010-2). The solutions that were selected for these studies are common nucleic acid extraction buffers and span a range of surface tension values. These solutions were GuHCl buffer (4 M guanidine hydrochloride, 25 mM sodium citrate, pH 7.0), 50% EtOH GuSCN buffer (50% ethanol, 2 M guanidine thiocyanate, 25 mM sodium citrate, pH 7.0), 80% EtOH buffer (80% ethanol, 5 mM potassium phosphate, pH 8.5), and deionized water. The surface tension valve fluids used in these studies include air or molecular biology grade mineral oil (Bio-Rad). A 2.54 cm cube magnet (Emovendo, SKU # M1CU) was used to transport the magnetic beads through the solutions in the tubing.

The range of surface and interfacial tensions of the solution/valve interfaces used in these studies spans from ∼0 to 72 dyn/cm, and the range of surface energies of the tubing tested spans the range of commercially available materials (∼20 to 42 dyn/cm) (Figures 3(a) and 3(b)). Photographs comparing the configurations used in these studies are shown in Figure 3(c). The most notable difference among the tubes is the curvature of the menisci, which reflect the large range of solid/liquid/gas interactions evaluated in these studies. Tubes were prepared by loading them with solutions serially through one end of the tube using a pipette. Unless otherwise noted, the baseline experimental configuration for these studies is an 8 cm length of 1.6 mm i.d. Tygon R-3603 tubing preloaded with two 75 μl water aliquots separated by a 1 cm air gap (valve). The tubing was plugged on both ends using plastic end caps. Solution carryover and magnetic force studies were performed using 1 mg of Dynabeads MyOne Silane.

B. Surface energy measurements

The surface energy of the materials used in these studies was calculated using the Zisman method. Contact angles of several test liquids spanning a range of surface tensions, including distilled water, glycerol, formamide, ethylene glycol, 1-bromonaphthalene, and diiodomethane, on

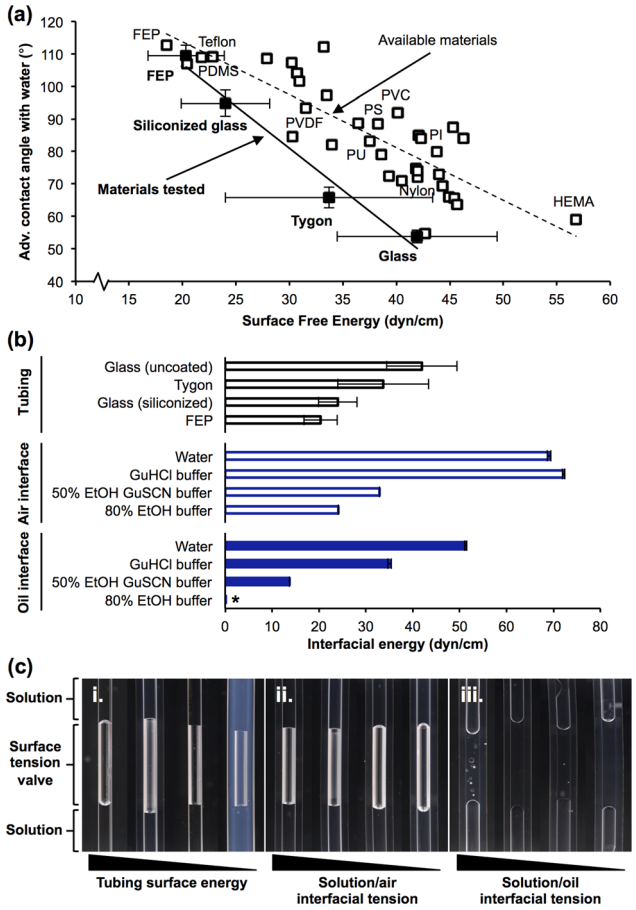

FIG. 3. The properties of the materials tested in these studies span a wide range of values. (a) The surface free energy of the various materials is related to the advancing contact angle with water. The surface free energies of the materials used in these studies (solid squares) span the range of available materials (open squares). (b) Tubing, solutions, and valve types tested span a wide range of interfacial energies. (c) Images showing the curvature of the menisci for tubing materials, solutions, and valve fluids evaluated in these studies. From left to right: (i) water and an air valve in tubing of decreasing surface energies. (ii) Tygon tubing and an air valve with solutions of decreasing interfacial tensions. (iii) Tygon tubing and a mineral oil valve with solutions of decreasing interfacial tensions.

the surface of each material were recorded using a standard goniometer (Rame-Hart Inst., model 200-F4). Three angle measurements were taken from four separate $\sim 2\,\mu$l drops placed on each material. The surfaces of the materials were cleaned after each test liquid by rinsing with deionized water and then 100% ethanol. The surface energy of each material was calculated using the Zisman plots created using the DROPIMAGE Standard V. 2.4 software (Rame-Hart Inst.).

C. Interfacial tension measurements

Interfacial tension of the solutions used in these studies was determined by using a Simga 700 Tensiometer (Biolin Sci.) using the Du Nouy ring method. The Du Nouy ring was lowered into 30 ml of each test liquid and the force data was collected and analyzed using the Attension Sigma software (Biolin Sci.). Each measurement was repeated 25 times for each solution. For interfacial surface tension between the liquids and mineral oil, the same protocol was followed except that 30 ml of mineral oil was layered on top of each solution tested, and the ring was lowered into the mineral oil layer before measurements were made. The ring was washed thoroughly after each test with 100% ethanol. The readings through the layers of 80% EtOH buffer and mineral oil were approximately zero, because the two solutions swirled together during the measurements. Therefore, the interfacial tension of the 80% EtOH buffer interfaced with mineral oil was approximated as 0 dyn/cm.

D. Valve stability measurements

A centrifuge suitable for spinning the tubes was constructed to produce an effective acceleration, or body force, in the x, y, or z direction of the tube. The centrifuge was used for measuring the relative centrifugal force (RCF) at which the surface tension valve fails. A motor interfaced with LABVIEW software was used to spin the tubes containing the surface tension valve at defined speeds, which were converted to RCF through the following relationship: $\mathrm{RCF} = 1.12r\left(\frac{\mathrm{RPM}}{1000}\right)^2$. Tubes were prepared by loading a solution containing Brilliant Blue dye on one side of a surface tension valve with a solution containing no dye on the other side of the valve. Rotational velocity was gradually increased until the valve failed as defined by blue color appearing in the clear solution on the opposite side of the valve. The stability values are reported in terms of the g-force that caused the valve to fail when applied in the direction perpendicular to the tubing wall, which is the orientation that is most likely to cause the valve to fail. The effects of the properties of the surface tension valves on valve failure were determined for different tubing types and diameters, valve contents and lengths, and solution contents. This method was validated using an impact-based drop method to evaluate valve stability.[16] The baseline configuration used in these studies was 1.6 mm i.d. Tygon tubing loaded with two 75 μl volumes of water separated by an air valve, unless otherwise noted.

E. Valve penetrability measurements

The force required to move a group of beads through the surface tension valve in the linear tubing, where the movement is constrained to the x direction only ($\mathrm{F_{m,}}_x$), was calculated using the following equation described by Gijs:[17]

$$\mathrm{F_{m,}}_x = \frac{V\chi_v}{\mu_o}\left(B_x\frac{\partial}{\partial x} + B_y\frac{\partial}{\partial y} + B_z\frac{\partial}{\partial z}\right)B_x,$$

where V is the volume occupied by the magnetic beads (m^3), χ_v is the volume susceptibility (CGS), μ_0 is the permeability of free space ($4\pi \times 10^{-7}$ T·m/A), and B is the magnetic field along the axis of the tube through which we are pulling the beads (T/m). Volume (V) was measured as the bulk volume that the particles occupied under the influence of a magnetic field. The values were calculated by measuring the cylindrical volume that a known mass of beads occupied in a short length of 1.6 mm i.d. tubing. Volume susceptibility (χ_v) of the magnetic particles was calculated by measuring the magnetic susceptibility of the beads using an Alfa Aesar Magnetic Susceptibility Balance Mark 1. This was done by taking 1 mg of Dynal beads, MagAttract beads, or Bioneer beads and diluting them into 114 mg silica gel, which is the amount required to fill the standard size glass tubes to the required ~3 cm height. The calibration constant was calculated using the manganese chloride standard supplied by the manufacturer. The blank was made using 114 mg silica gel without beads added. The tube was rinsed with water between each sample, dried at 100 °C for 10 min, and the magnetic susceptibility of the empty tube was measured to verify that residual magnetic beads had been removed after each wash. Each sample was measured three times, removing and repacking the beads between each measurement. Mass susceptibility (χ_g) was calculated using the following equation:

$$\chi_g = \frac{\mathrm{C}_{bal} \times (\mathrm{R} - \mathrm{R}_o) \times l}{10^9 \times m},$$

where C_{bal} is the calibration constant, R is the sample value, R_0 is the blank value, l is the length (cm) of sample in tube, and m is the mass of magnetic sample in the tube. This was converted to volume susceptibility (χ_v) using the following conversion factor: $\chi_v = \chi_g d$, where d is the bulk density of the beads in the presence of a magnetic field.

To measure the force required to pull the beads through the solution/valve interface, an apparatus was developed to measure x, y, and z coordinates of the magnetic field (B) of a 2.54 cm cube permanent magnet (Emovendo, SKU# M1CU) at 0.5 mm intervals along the axis of the

tube using a F.W. Bell series 9900 Gaussmeter. The values for the x, y, and z coordinates were plotted as a function of distance from the edge of the magnet. The gradient of the magnetic field for the x, y, and z coordinates $\left(B_{(x,y,z)}\frac{\partial}{\partial(x,y,z)}\right)$ was approximated using the slope of the lines between two consecutive magnetic field measurements. Because the gradient of the magnetic field in the y and z coordinates was approximately zero, the $B_y\frac{\partial}{\partial y}$ and $B_z\frac{\partial}{\partial z}$ terms of the magnetic force equation were set to zero.

To measure the force required to pull the beads through a surface tension valve, a preloaded tube containing magnetic beads was slowly moved toward the 2.54 cm cube magnet along the x coordinate of the measured magnetic field until the point at which the beads pulled through the valve interface. The distance of the interface from the magnet was recorded and used to approximate the magnetic field strength (B_x) and the magnetic field gradient ($B_x\frac{\partial}{\partial x}$) at that distance. The magnetic force requirement values for 1 mg Dynabeads MyOne Silane beads moving from water into an air valve in Tygon R-3603 tubing were validated using a second permanent magnet, one-forth the size of the magnet described above (1.27 cm cube), similar to the methods described above.[16] The baseline experimental configuration used in these studies was 1.6 mm i.d. Tygon tubing loaded with 75 μl volume of water and another 75 μl volume of water containing 1 mg Dynabeads MyOne Silane beads separated by an air valve, unless otherwise noted.

F. Solution carryover measurements

Carryover volume was measured using a fluorescence-based assay. In these studies, fluorescein was added to each solution tested and standard curves were made for small volumes of each solution diluted into water. The standard curves for each solution had R^2 values >0.98 and consisted of at least five data points. Tubes were loaded with a test solution containing fluorescein and with water separated by an air or mineral oil valve. Using a permanent magnet, beads were pulled from the fluorescein-containing solution, through the valve, and into the water solution. Then the beads were mixed with the water and removed from the water. The amount of liquid carryover associated with the beads was measured by plotting the value of fluorescence that was introduced into the water on the standard curve of fluorescein in the corresponding solutions diluted into water. The effect of material properties on solution carryover was determined for different tubing types, valve contents and lengths, bead types and masses, and solution contents. The solution carryover values for 1 mg Dynabeads MyOne Silane beads moving from water into an air valve in 1.6 mm i.d. Tygon R-3603 tubing were validated using a solution mass measurement.[16] The baseline experimental configuration used in these studies was 1.6 mm i.d. Tygon tubing loaded with 75 μl volume of water and another 75 μl volume of water containing 1 mg Dynabeads MyOne Silane beads separated by an air valve, unless otherwise noted.

G. Imaging

Digital photographs of the fluids within the tubing for the various configurations tested were collected using a Nikon D100 D-SLR camera with a 60 mm AF Micro Nikkor lens and three Kenko extension tubes (58 mm total extension length). Videos of the magnetic beads crossing the surface tension valves were recorded using a Nikon D800 D-SLR camera with the lens and extension tubes used for collecting the images.

Electron micrographs of the three bead types were collected using a Hitachi scanning electron microscope at 3000× zoom with a 3 kV beam strength, a working distance of 14 mm, and an objective aperture position of 2. Bead samples were prepared by pipetting 5 μl of each bead suspension directly onto an aluminum specimen mount and drying at 80 °C overnight.

III. RESULTS

In experimental evaluations of the self-contained format based on surface tension valves we sought to identify physical parameters which (i) maximized valve stability, (ii) enhanced

valve penetrability by magnetic beads, and (iii) minimized the carryover of one processing solution to the next. Each of these characteristics is critical for developing an assay format that is simple, robust and effective outside of a laboratory setting. The greatest utility is achieved with a stable preloaded assay format, which could be transported and stored for long periods of time. Similarly, enhancing valve penetrability minimizes the magnetic force required for processing and yields the most effective format for reproducible assay results. And finally, carryover between processing steps is minimized as it can contaminate and negatively impact downstream chemistries and molecular interactions. Factors that affect each of these performance characteristics are detailed in the following sections.

A. Valve fluid stability

Tubing diameter has a substantial effect on valve stability. Valves prepared in the smallest commercially available diameter of Tygon R-3603 tubing (0.8 mm i.d.) were extremely stable, and did not fail at 84.9 g (the highest RCF tested), which is equivalent to dropping the tubing from a height of ~8.4 m, assuming no air drag (Figure 4(a)). The stability drops off exponentially with increasing tubing diameters up to 4.8 mm (surface tension valves can not be supported in tubing with a 5.6 mm diameter or greater). The effect of the surface energy of the tubing material was not as striking but suggests a linear, inverse relationship between valve stability and tubing surface energy (Figure 4(b)). Valves prepared in FEP tubing, which has the lowest surface energy of the tubing materials tested (20.3 dyn/cm), were the most stable, failing at 48.2 g. Valves prepared in glass tubing, which has the highest surface energy of those tested (42 dyn/cm), on the other hand, were the least stable, failing at 22.5 g.

Valve fluid also had a substantial effect on valve stability. Overall, valves prepared with mineral oil were significantly more stable than those prepared with air (Figures 4(c) and 4(d)). Additionally, mineral oil valve stability decreased, whereas air valve stability increased, with increasing interfacial tension. The most stable valve tested under these conditions was mineral oil interfaced with 80% EtOH buffer, which did not fail at the highest RCF tested (80 g), whereas the least stable valve was air interfaced with 80% EtOH buffer, which failed at 4.5 g (Figure 4(c)). The density difference between the solution and valve appears to be somewhat predictive of valve stability for all the solution/valve combinations tested, particularly for solutions interfaced with mineral oil valves (Figure 4(d)).

The length of the valve and the volume of the processing solutions on valve stability were also evaluated. Tygon R-3603 tubing (1.6 mm i.d.) was loaded with a range of air valve lengths up to 2 cm separating the two water solutions. We found that the smallest possible air valve, or separation gap, that can effectively prevent solution intermixing was 0.15 cm, which was slightly less stable than a 0.3 cm valve length, most likely because the opposing menisci of adjacent solutions are nearly touching. All valve lengths 0.3 cm or longer failed at approximately 29 g, suggesting that air separations longer than 0.3 cm offer no advantage for valve stability (Figure 4(e)). All solution volumes tested (10–90 μl water) had approximately the same stability, failing at approximately 29 g (Figure 4(f)). Although the tested range of valve lengths and solution volumes was limited by the design of the centrifuge, it is reasonable to assume that valve lengths and solution volumes greater than those tested would follow the established trend and also have little influence on the stability.

B. Valve penetrability

The force required to pull the beads from water into the air valve was much greater than the force to pull from the air valve into water (372 ± 78 μN and 52 ± 8.7 μN, respectively) (Figure S1).[16] A similar trend was observed using a mineral oil valve (18.7 ± 8.2 μN and 1.6 ± 0.3 μN, respectively), although overall the forces were significantly smaller than those with the air valve. Additionally, the force require to pull beads along the tubing wall within an aqueous solution (i.e. the force required to overcome friction and drag) is the same as the force required to pull beads from an oil valve into water (1.6 ± 0.3 μN), which is negligible compared to the forces required for beads to penetrate the solution/valve interface. Therefore, the force

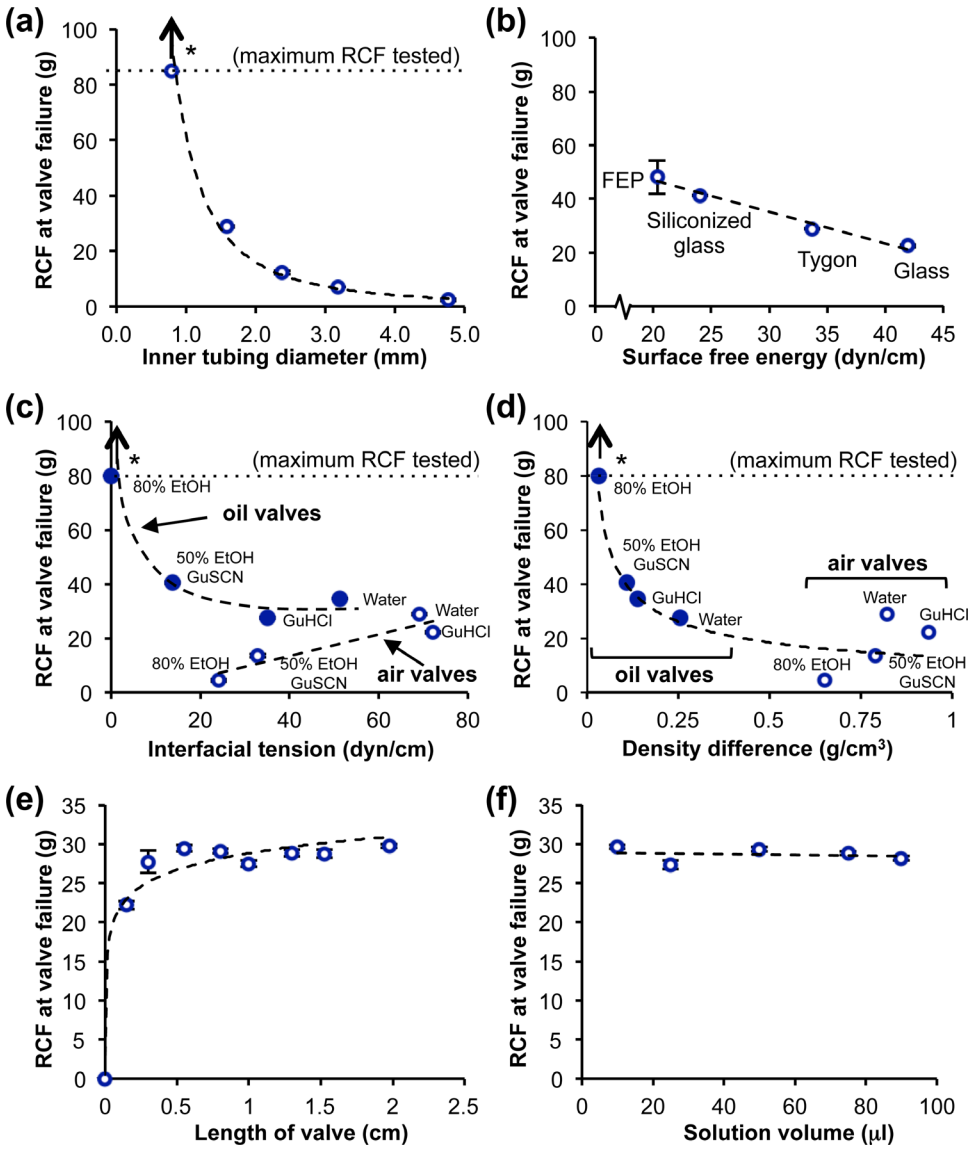

FIG. 4. The effect of material properties on the stability of the surface tension valve. (a) Surface tension valves within tubing with smaller inner diameter are much more stable than those within tubing with larger diameters. (b) Surface tension valves within tubing with low surface energy are more stable than those within tubing with high surface energy. (c) The stability of mineral oil valves decreases with increasing interfacial tension (solid circles), whereas the stability of air valves increases linearly with increasing interfacial tension (open circles). (d) Surface tension valves interfaced with solutions of similar density are much more stable than those interfaced with solutions with a greater difference in density. (e) Valve stability increases sharply with valve lengths smaller than 0.3 cm and remains consistent with longer valve lengths. (f) The volume of water flanking the valve has little effect on the stability of the valve. The symbol * indicates that the valve did not fail at maximum RCF tested. (n = 3, mean ± s.d.; if not visible, error bars are obscured by the symbols)

required to pull the beads from the solution into the valve, through the solution/valve interface, is reported, since this is the largest of the forces and thus the limiting force for transporting beads.

The diameter of the tubing had a significant impact on valve penetrability. The force required to pull the beads through an air valve drops significantly with larger diameter tubing (Figure 5(a)). In 0.8 mm i.d. tubing, the required force is large and variable (678 ± 271 μN), whereas in larger tubing diameters (2.4 to 4.8 mm i.d.) the required forces are much lower, in the range of ~60 to 100 μN. The effect of tubing surface energy was less conclusive. With the exception of Tygon R-3603 tubing, which has a force requirement of 326 ± 78 μN, there

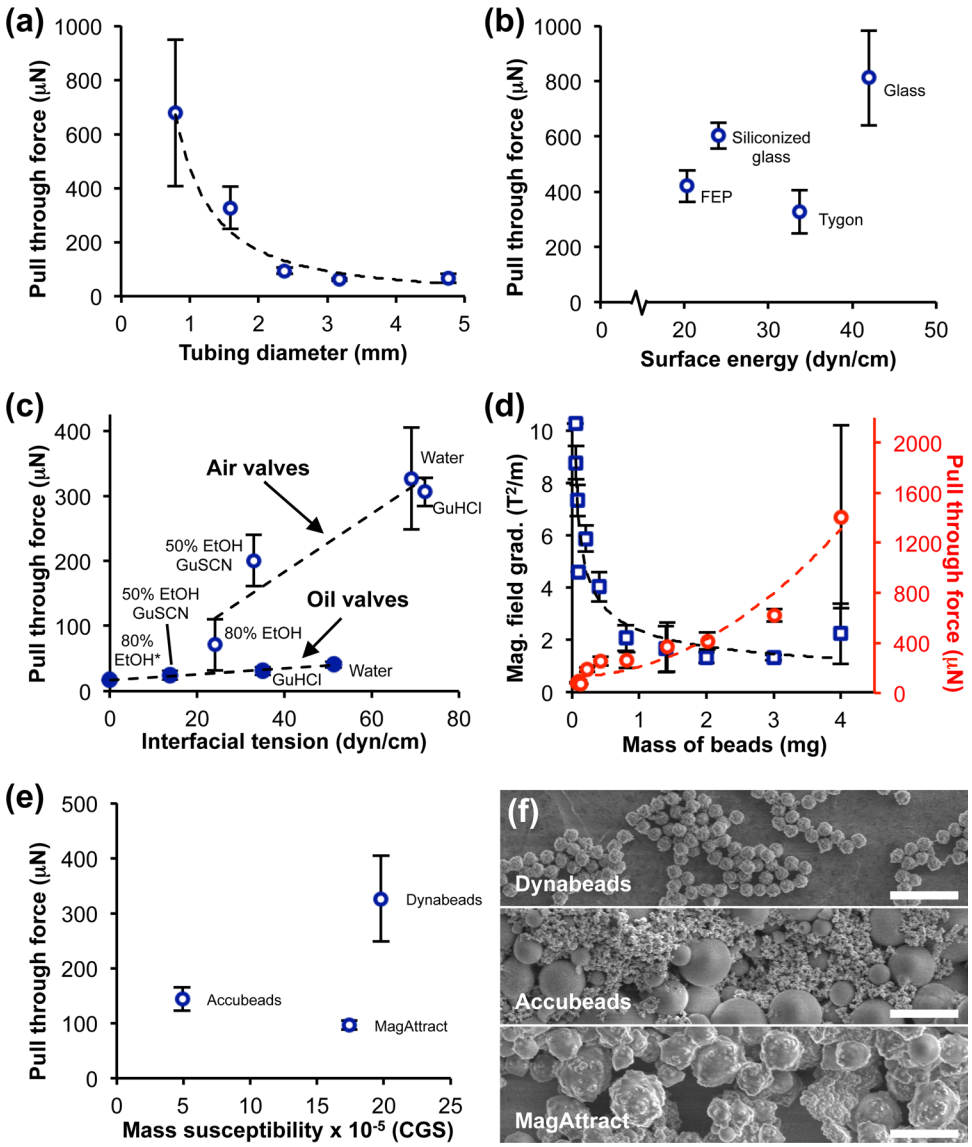

FIG. 5. The effect of material properties on force required to pull beads through the solution/valve interface. (a) The force required decreases when using tubing of a larger diameter. (b) With the exception of Tygon tubing, the force required to pull beads across a surface tension valve increases with the surface energy of the tubing. (c) The force required to pull beads through mineral oil valves (solid circles) is significantly less than the force required to pull beads across air valves (open circles). Force required increases with interfacial tension with both types of valves. (d) The magnetic field gradient along the x axis that is required to pull the beads through the valve (squares) increases with the amount of beads, whereas the magnetic field required (circles) decreases. (e) The mass susceptibility of the bead used has little influence over the force required to pull beads across a surface tension valve. (f) Scanning electron microscopy images of the three commercially available silica-coated magnetic beads (scale bars = 5 μm). (n = 3, mean ± s.d.; if not visible, error bars are obscured by the symbols)

appears to be a positive correlation between surface energy of the tubing and force required to pull the beads through the valve (Figure 5(b)). The tubing with the lowest surface energy (FEP) required a pull through force of 420 ± 58 μN, and the tubing with the highest surface energy (glass) required a pull though force of 812 ± 172 μN.

Overall, the force required to pull the beads through a mineral oil valve was significantly lower than the force required for air valves (Figure 5(c)). The force required to pull the beads through both mineral oil valves and air valves increased as the interfacial energy increased, though the increase was more substantial with air valves. The solution with the lowest

interfacial energy (80% EtOH buffer solution interfaced with mineral oil) had a force requirement of just $17 \pm 0.8\ \mu$N, whereas the solution with the highest interfacial energy (water interfaced with air) required $326 \pm 78\ \mu$N.

Interestingly, the force required to pull beads through the valve increased with increasing bead mass, whereas the magnetic field gradient required decreased (Figure 5(d)). The bead mass range that has the lowest magnetic field gradient requirement is between 1 and 3 mg. Bead masses less than 1 mg become increasingly difficult to pull across the solution/valve interface with 0.048 mg being the minimum bead mass that can be pulled through the interface under the baseline experimental conditions. Masses much more than 3 mg beads fill the entire diameter of 1.6 mm tubing and increase the experimental error.

The force required to pull three commercially available bead types was also investigated. Although one might expect that mass susceptibility would be inversely related to the force required for transport across a valve, there was no clear trend among the three types of beads tested (Figure 5(e)). It is interesting to note that despite having similar product descriptions, there was substantial variation in the morphology of these bead types as determined by scanning electron microscopy. The variability in force requirement appears to reflect their varying sizes and dispersity (Figure 5(f)): Dynabeads are relatively small, monodispersed ($1.1 \pm 0.07\ \mu$m) silica-coated magnetite spheres; Accubeads are relatively large, polydispersed ($2.4 \pm 1.7\ \mu$m) silica spheres trapped amidst magnetite crystals; and MagAttract beads are relatively large, polydispersed ($3.7 \pm 1.9\ \mu$m) and amorphous silica-coated magnetite.

C. Solution carryover

The number of beads used had the greatest influence on the amount of liquid carried across the valve (Figure 6). Using increasing amounts of Dynabeads MyOne Silane beads, the carryover volume increased proportionally for the mass of beads tested (Figure 6(b)). Water carryover is $\sim 1.5\ \mu$l per milligram of beads, which equals ~ 3.6 fl of water per bead, assuming that the beads are uniformly $1.15\ \mu$m in diameter, $3\ \text{g/cm}^3$ in density, and that there are 4.2×10^8 beads per milligram.

There was no clear relationship between surface free energy of the tubing or interfacial energy at the solution/valve interface to the carryover volume associated with the beads. The carryover volume using the four types of tubing was fairly similar and increased only modestly ($0.9–1.2\ \mu$l) as the surface energy of the tubing increased (Figure 6(c)). Overall, the carryover volume associated with beads pulled through mineral oil valves was higher than with beads pulled through air valves, except with the 80% EtOH buffer solution, which had the same amount of carryover with air and mineral oil (Figure 6(d), solid bars vs. open bars, respectively). The range of carryover volumes was $1.2–1.9\ \mu$l with air valves and $1.2–2.4\ \mu$l with mineral oil valves.

IV. DISCUSSION AND CONCLUSIONS

Magnetic bead-based methods developed for biomarker isolation, amplification, and detection would be especially relevant for diagnostics in low resource settings, but in many cases, they are not used because of the prevalence of environmental contaminants and the limited access to trained personnel. As the self-contained assay format described in this report and our previous work is sealed from the environment and does not require extensive solution handling or pipetting, it has the potential to facilitate the application of these magnetic bead-based assays in settings that lack laboratory resources.[9,10] Furthermore, as demonstrated by the experimental results of these studies, the multiphase fluidic-based format has the flexibility to handle the diverse constraints and requirements of a variety of magnetic bead assays.

The manipulation fluid-fluid interfaces for performing simplified and automated chemical and biological assays is of general interest, especially in the field of microfluidics. Consequently, the physical properties governing multiphase microfluidics are under investigation by many researchers. Some of the many aspects of the phenomena that control microdroplet formation were recently presented in a special collection of papers.[18–21] Interestingly, some of the

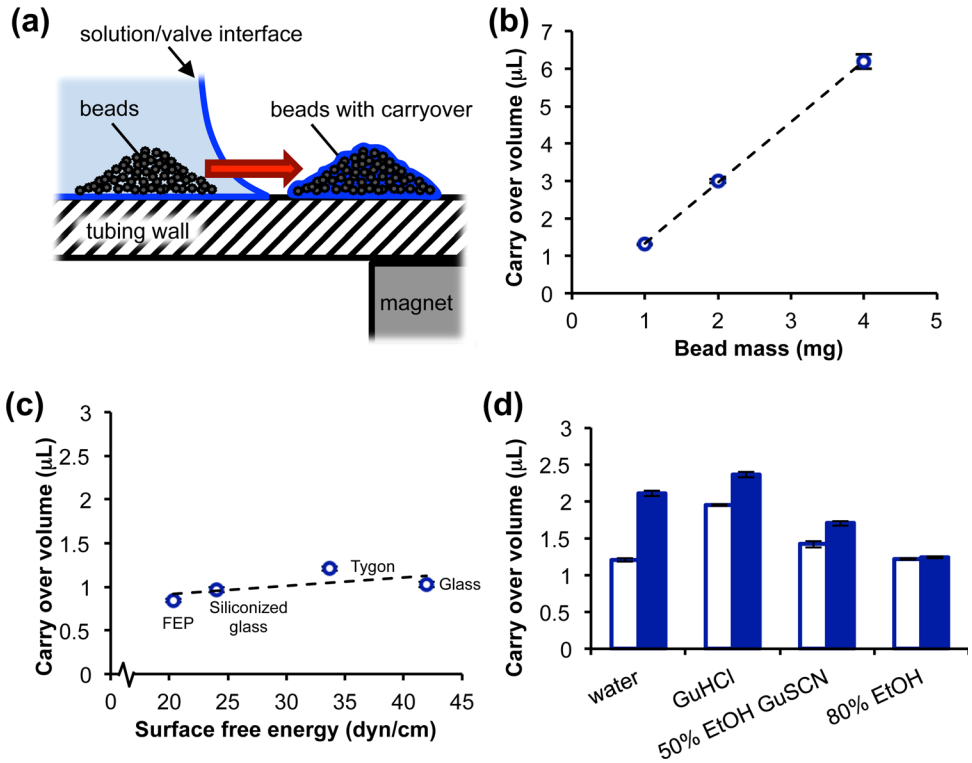

FIG. 6. The effect of the material properties on the amount of solution carryover between solutions. (a) Illustration of magnetic beads under the influence of a magnetic field moving from a solution through a surface tension valve within small-diameter tubing. As beads traverse the solution/valve interface, a small amount of solution is retained amidst the beads and is carried across the valve and into the next solution. (b) The carryover volume increases linearly with an increased number of beads. (c) The surface energy of the tubing has little effect on the carryover volume. (d) In all solutions except 80% ethanol, there is more carryover when using mineral oil valves (solid bars) compared to air valves (open bars). (n = 3, mean ± s.d.; if not visible, error bars are obscured by the symbols)

physical properties that influence fluid stability within our self-contained format, such as surface and interfacial tension and solution density difference at the fluid-fluid interface, are similar to those that govern microdroplet formation in multiphase microfluidics. In our system, additional variables and constraints associated with magnetic particles are discussed in the context of the multiphase fluidic system.

The self-contained sample processing format based on surface tension valves functions well because of three phenomena: (i) solutions arrayed in millimeter-diameter tubing and separated by immiscible fluid spacers remain isolated from one another, (ii) magnetic beads under a magnetic field gradient can be transported across the surface tension barrier of the fluid separators, and iii) magnetic beads passing between adjacent solutions through a surface tension interface do not intermix the solutions. Because of these phenomena, the tubing can be preloaded with processing solutions that are effectively separated by surface tension valves, and the assay can be carried out simply by moving the functionalized beads through the solutions using an externally applied magnetic field. The results of these studies outline the physical design constraints for which these phenomena remain true. Based on these results, this discussion presents a generalized strategy for reconfiguring magnetic bead assays to the self-contained format.

The optimal design for the self-contained format maximizes valve stability, minimizes the force required to pull beads through the valves, and minimizes the solution carryover across the valve. Because the most useful relationships from a design standpoint are between valve stability and penetrability, the results for all the parameters tested in these studies are plotted in terms of their effects on these two performance characteristics (Figure 7). Valve stability is expressed in terms of a modified form of the Bond number ($\Delta\rho r^2 g/\sin\theta\gamma$), where $\Delta\rho$ is the difference in density across the valve interface (g/m^3), r is the radius of the tubing (m), g is the

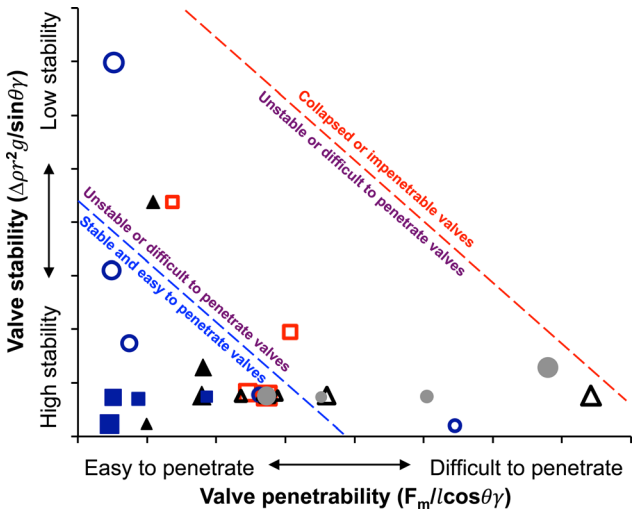

FIG. 7. Comparison of the valve fluid stability and penetrability for various material configurations. Solutions interfaced with mineral oil valves (solid squares) are more stable and easier to penetrate than solutions interfaced with air valves (open squares). There are tradeoffs between stability and penetrability with the range of tubing diameters tested (open circles). Tubing surface energy (solid circles) and bead mass (open triangles) influence valve penetrability but not valve stability. The minimum mass of beads that can be pulled through a valve (solid triangles) can be optimized for valve stability and penetrability. The relative size of the symbols corresponds to the relative material property values (e.g., large solid squares have higher interfacial energy than smaller solid squares). The positions of the zone boundaries indicated by the dotted lines are approximations.

gravitational acceleration constant (9.8 m/s^2), θ is the contact angle of the solution on the tubing wall (°), and γ is the interfacial tension at the solution/valve interface (N/m). The Bond number is a dimensionless relationship of the accelerative forces and the surface tension forces that determine whether the surface tension valve maintains the separation between two adjacent solutions in small diameter tubing. The Bond number is generally used to determine the stability of drops suspended in free solution. For these studies, we have modified the Bond number to make it more appropriate for the configuration of our fluids, which are interfaced with the solid surface of the inner tube wall. In our configuration, the gravitational acceleration acts opposite that of the vertical component of the interfacial tension, so by including the $\sin\theta$, only the vertical component of the interfacial tension is considered. Valve penetrability is expressed in terms of the Penetrability number ($F_{m,x}/l\cos\theta\gamma$), where $F_{m,x}$ is the force required to move a group of beads the x direction only through the surface tension valve interface (N) (see Sec. II E), l is the contact line of the group of beads on the solution/valve interface (m), θ is the contact angle of the solution on the tubing wall (°), and γ is the interfacial tension at the solution/valve interface (N/m). The Penetrability number is a dimensionless number that we have developed to describe the relationship between the magnetic forces and surface tension forces that act on the beads to determine whether the magnetic beads cross the solution/valve interface. Plotting the modified Bond number versus the Penetrability number is useful for identifying configurations that support a surface tension valve that is both highly stable and easy to penetrate with magnetic beads. For reference, the values that reflect the configuration used for our previously published RNA extraction assay (see Ref. 9) are represented as the red open square symbols (see Figure 7). Plotting the modified Bond number versus the Penetrability number is also useful for determining the effect of changing a single parameter on these performance characteristics, and it can be utilized for identifying variables that can be manipulated when one variable is constrained by a particular internal or external constraint. The parameters that influence the stability and/or penetrability to the greatest degree and are therefore the most important to optimize, span a range of values outside of the region identified as stable and easy to penetrate (Figure 7). In these studies, those parameters include tubing diameter, tubing surface energy, and bead mass (open circles, closed circles, and open triangles, respectively).

When reconfiguring a magnetic bead-based assay into the self-contained format, it is necessary to balance the physical design constraints of a configuration within the context of the chemical constraints of the assay, as the chemical composition of the processing solutions is connected to the function of a particular assay. For example, the surface tension and density of the processing solutions are intrinsically associated with the assay performance and are generally unalterable constraints for designing the physical format of the device. Consequently, an important step to designing a self-contained format for a particular assay is to identify the internal constraints of the assay. Internal constraints include the arrangement and volumes of the solutions, which influence the overall length of the tube; the compositions of the reagents, which influence the surface tension of the solutions; and the analyte-binding capacity of the beads, which influences the amount of beads used. These factors must be taken into account when choosing materials for configuring the self-contained format, as the physical configuration for optimizing one performance indicator can conflict with the configuration for optimizing another.

It is clear that tubing material and diameter strongly influence the physical performance characteristics of this assay format. In general, tubing material of the lowest surface energy (i.e., the most hydrophobic) is preferred as it reduces the magnetic force required to pull beads through the valve (Figure 5(b)) and, to a lesser extent, increases the stability of the surface tension valve (Figure 4(b)). The choice of tubing diameter, on the other hand, must be considered as a tradeoff between valve stability and penetrability (see Figures 4(a), 5(a), and 7). Small diameter tubing maintains a stable valve but requires a very high magnetic force to move beads across the valve. The opposite is true with large diameter tubing, where the valves become less stable as the magnetic force requirement is minimized. The Penetrability number described above can explain these phenomena. The surface energy of the tubing material determines the contact angle (θ) of the solution at the tubing wall. With lower material surface energy, the contact angle increases, which in turn decreases the Penetrability number (i.e., makes the surface tension valve easier to penetrate). Tubing diameter on the other hand, influences the contact line (l) of the group of beads on the solution/valve interface. With larger tubing diameters the contact line increases, which also decreases the Penetrability number. The use of 1.6 to 2.4 mm i.d. tubing with low surface energy appears to be optimal for maximizing both stability and penetrability. In contrast, valve length and solution volume within the ranges most likely to be used in biological assays, do not influence valve stability or penetrability (Figures 4(e) and 4(f) and data not shown).

Another important design criterion is the immiscible fluid used to separate the processing solutions within the tubing. The fluid must provide an adequately stable barrier between solutions and permit the transit of the magnetic beads through the interface. We have found that separating processing solutions with air works well for practical reasons. Surface tension valves made from air are easier to load and more reproducibly separate solutions in millimeter-diameter tubing compared to those made with mineral oil, as mineral oil tends to inconveniently adhere to the surface of the tubing. Nevertheless, mineral oil works well for maximizing stability and minimizing bead pull through force (Figures 4(c) and 5(c), respectively). One of the most stable configurations tested was the use of the 80% EtOH buffer solution separated by a mineral oil valve. This combination is interesting as the 80% EtOH buffer solution has nearly the same density as the mineral oil valve used in these experiments (0.83 and 0.86 g/cm^3, respectively). The modified Bond number can explain the high stability of this valve configuration. One of the variables represented in the modified Bond number is the difference in density between the fluids at the valve interface ($\Delta\rho$). Because there is such a low difference in density between the solution and the valve (0.03 g/cm^3), the body force (g) acts on each fluid almost equally. This results in a lower value for the modified Bond number and a more stable solution/valve interface. Difference in density, however, is not predictive of valve stability with air valves (Figure 4(d)). It appears that with a liquid surface tension valve (i.e., mineral oil) interfaced with adjacent solutions, the density difference between the solution and valve has a dominating influence on valve stability, and with a gas surface tension valve (i.e., air) interfaced with adjacent solutions, the surface tension has a dominating influence on stability. Despite the convenience and reproducibility of air separators for

preloading processing solutions, applications that require high valve stability or valves that are easy to penetrate may benefit most from mineral oil valves.

Magnetic beads of an adequate mass under the influence of a sufficient magnetic field gradient can overcome surface tension barrier of the fluid separators and traverse the surface tension valve. A low magnetic force requirement is ideal, because the use of a small permanent magnet or an electromagnet is most desirable for the development of automated assay formats where limitations on magnet size or power requirements may exist. The force required to pull beads through the valve increased with increasing bead mass (Figure 5(d)). This positive correlation between pull-through force and bead mass was also observed by Shikida *et al.*[15] Interestingly, while the force required increased with increasing bead mass, the magnetic field gradient required decreased (Figure 5(d)). This is because a lower magnetic field gradient produces a much larger force on beads of increasing mass, as the force acting on the beads is directly proportional to both magnetic field gradient and bead mass. We have found that valve penetrability is maximized using a mass of approximately 1–3 mg beads in 1.6 mm i.d. tubing (Figure 5(d)). The minimum mass of beads that penetrated the water/air interface in 1.6 mm Tygon tubing and a magnetic field gradient of $\sim$10.2 T^2/m was 0.048 mg. Because the magnetic force acting on the beads is directly related to bead mass, the magnetic force that can be generated for bead masses below this minimum threshold are not sufficient to overcome the surface tension forces of the meniscus. This minimum bead mass value is in the range of those determined by Shikida *et al.* using beads of much larger diameters.[15] It was, however, observed that bead masses less than 0.2 mg require more time and effort to pull through the solution/valve interface. Some magnetic bead-based assays may require the use of low amounts of beads (i.e., $<$0.2 mg) to optimize the surface area available for binding the biomolecules of interest while limiting nonspecific binding of nonspecifically bound contaminants. Therefore, increasing the number of beads in a particular assay to reduce the magnetic field gradient required to pull the beads through a valve may have deleterious effects on the assay. We have observed, however, that the Qiagen MagAttract beads required the least amount of force to move through the surface tension valves (Figure 5(e)), likely because of the relatively large size of the individual beads (see Figure 5(d)). Because the surface area to bead mass ratio decreases as the diameter of the beads increases, the use of larger beads may resolve this potential problem because of their lower surface area to mass ratio. Another way to facilitate valve penetration in the case that small masses of beads are to be used is to reduce the surface tension at the interface using a detergent. In a recent report of a 96-well plate mRNA extraction assay analogous to our continuous tubing design, Berry *et al.* use low concentrations of Triton X-100 to reduce the interfacial tension at the solution/valve interface.[13] The group reported that the addition of 0.01–0.1% Triton X-100 did not interfere with the mRNA binding chemistry yet facilitated the magnetic transfer of the beads across the immiscible phase.

Although the integrity of the valve is maintained and the solutions do not intermix when beads traverse a surface tension valve, a relatively small volume of solution associated with the magnetic particles is carried to downstream solutions. Minimizing solution carryover is most desirable for the majority of bead-based assays, because it limits the amount of nonspecifically associated species that are carried over in the solution surrounding the beads that may interfere with the efficacy of the chemistry of the downstream solutions. In the case of the nucleic acid biomarker extraction assays developed in our laboratories and others, it has been reported that the carryover of GuSCN, GuHCl, or ethanol from upstream solutions into the eluate can negatively impact the polymerase chain reaction (PCR).[14] The results of these studies indicate that the smallest amount of solution carryover is achieved with the fewest beads possible, as the amount of carryover is proportional to the number of beads used. Tubing surface energy, solution interfacial energy, and valve fluid had less impact on carryover; all carryover volumes fell between 1 and 2 μl per milligram of beads for each configuration tested when using 1 mg Dynabeads (Figures 6(c) and 6(d)), which represents between 0.3% and 4% of the processing solution volumes used in the most common nucleic acid extraction assays. Notably, this carryover volume is approximately equivalent to that of the commercially available Dynabeads Silane viral NA (Invitrogen) kit.

We have shown that this simple, self-contained format functions well for a variety of biomarker extraction assays.[9,10] This format has many advantages for implementation in low-resource settings compared to laboratory-based assays. Foremost is the simplicity of the preloaded cassette. Because the tubing can be preloaded with assay solutions, the processing steps are self-contained, which reduces the potential for contamination during the assay with the use of an externally applied magnetic field to move the functionalized beads. The self-contained format also has the flexibility to interface with other assays, as the tubing permits direct injection or coupling to upstream and downstream systems for introducing or removing samples, reagents, or products. Furthermore, automated and multiplexed processing could be achieved by simply manipulating the magnetic field gradient using electronic motors and/or electromagnets. Because of these advantages, this self-contained format may be extended to simplify a variety of magnetic bead-based assays that have potential diagnostic applications for low-resource settings.

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